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Achromatopsia as a Potential Candidate for Gene Therapy

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Abstract

Achromatopsia is an autosomal recessive retinal disease involving loss of cone function that afflicts approximately 1 in 30,000 individuals. Patients with achromatopsia usually have visual acuities lower than 20/200 because of the central vision loss, photophobia, complete color blindness and reduced cone-mediated electroretinographic (ERG) amplitudes. Mutations in three genes have been found to be the primary causes of achromatopsia, including *CNGB3* (beta subunit of the cone cyclic nucleotide-gated cation channel), *CNGA3* (alpha subunit of the cone cyclic nucleotide-gated cation channel), and *GNAT2* (cone specific alpha subunit of transducin). Naturally occurring mouse models with mutations in *Cnga3* (*cpfl5* mice) and *Gnat2* (*cpfl3* mice) were discovered at The Jackson Laboratory. A natural occurring canine model with *CNGB3* mutations has also been found. These animal models have many of the central phenotypic features of the corresponding human diseases. Using adeno-associated virus (AAV)-mediated gene therapy, we and others show that cone function can be restored in all three models. These data suggest that human achromatopsia may be a good candidate for corrective gene therapy.

1.1 Human Achromatopsia

The human retina has approximately 6 million cone photoreceptors and 100 million rod photoreceptors. Cones are primarily responsible for central, fine resolution and color vision while operating in low to very bright light; they are primarily concentrated in the central macula comprising nearly 100% of the fovea. In contrast, rods are responsible for peripheral, low light and night vision, and are primarily found in the peripheral retina and perimacular region. Achromatopsia, or rod monochromatism, is a recessive genetic condition characterized by cone dysfunction, thus leaving the patient with only rod mediated vision. There are two clinical forms of achromatopsia: complete and incomplete. The complete form results in serious visual deficits and affects approximately 1:30,000 Americans (Kohl et al. 2002). Those achromats exhibit total color vision loss, relatively stable central vision loss, and visual acuity of 20/200 or worse (Kohl et al. 2005), usually making them legally blind. Since these individuals see the world only with their rods, they experience photophobia or “daytime blindness” because their rods become light-saturated in normal bright light conditions.

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1.2 Clinical manifestations

Clinically, the first signs of achromatopsia in infants are the presence of nystagmus, a pendular quivering of the eyes, and photophobia as evidenced by squinting in bright light (Kohl et al. 2005). Infants can be tested by the cone ERG, and, as they mature they can be given specific tests such as the Sloan Achromatopsia Test.

1.3 Current achromatopsia treatments

There are no treatments available that correct cone function in achromats to any degree. Current standard of care consists of managing symptoms by limiting retinal light exposure with tinted contact lenses (Park et al. 2004), and (or) very darkly tinted sunglasses (Young, Krefman & Fishman 1982; Young, Krefman and Anderson 1983). Tinted central contact lenses typically transmitting light at wavelengths between 400–480nm (Park et al. 2004) are an improvement over simple cutoff filters in alleviating photophobia (Schornack 2007). Additionally, these contacts have reduced the stigma of wearing dark wraparound sunglasses indoors. However, such red central contacts only reduce the amount of light entering the retina and do nothing to improve high-resolution vision or color sensitive tasks. A partial solution is to use tinted contact lenses with magnification to boost what central vision is still present from functional photoreceptors (Fonda and Thomas 1974). With respect to visual acuity, improvement can be obtained by employing microscopic eyewear, enlarged print and closed circuit TV. Even with the best external aid techniques in place, daily tasks such as driving and going to school present significant obstacles. This is especially problematic for school age children because of the usual color-based curriculum; in this context tinted lenses have been tested (Schiefer 1995). A common result of these symptoms is that achromats often gravitate towards activities normally performed in the evening or in low light.

2.1 Genetics of human achromatopsia

Autosomal recessive mutations in predominantly three genes, *CNGB3, CNGA3*, and *GNAT2*, cause achromatopsia (Kohl et al. 1998, 2000, 2002, 2005; Sundin et al. 2000; Aligianis et al. 2002). CNGB3 encodes the beta subunit of the cone cyclic nucleotide-gated cation channel and *CNGA3* encodes the alpha subunit. *GNAT2* encodes the cone specific alpha subunit of transducin. Of all mutations that cause complete achromatopsia, those in *CNGB3* account for about 50% of cases whereas *CNGA3* mutations account for about 23% and *GNAT2* mutations for about 2% (Wissinger et al. 2001; Kohl et al. 2002, 2005; Aligianis et al. 2002). The majority of *CNGB3* mutations result no protein or protein truncations that are functionally null (Kohl et al. 2005). In contrast, *CNGA3* mutations are predominantly missense (Wissinger et al. 2001; Kohl et al. 2005). Recently, a Japanese patient with congenital achromatopsia was identified with compound heterozygous mutations in *CNGA3* (Goto-Omoto et al. 2006).

2.2 *GNAT2* achromatopsia

Genetic analysis points to several classes of mutations in the *GNAT2* gene that give rise to the similar phenotypes. Kohl et al. (Kohl et al. 2002) analyzed 77 achromatopsia patients with *GNAT2* mutations and identified six distinct disease-related sequence alterations that segregated in five apparently independent families of European descent. There was one nonsense mutation, four small deletion and/or insertion mutations and a sixth mutation containing a large intragenic deletion of 2,019 bp including exon 4 and flanking intron sequences. All mutations resulted in premature translation termination or in mutant polypeptides that lack considerable portions of the carboxy1 terminus. In rods the conserved carboxyl terminus of the corresponding rod α-transducin contains major sites of interaction with photo-excited rhodopsin (Cai, Itoh and Khorana 2001). Considering the high

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conservation between the rod and cone transducin α-subunits, a similar structural function is likely to exist in the cone system, and thus explain the carboxy-terminal mutations. Accordingly, Kohl et al. (Kohl et al. 2002) suggest that all mutations in these achromatopsia families represent effectively null alleles of *GNAT2*, which prevent either the formation of a functional heterotrimeric G-protein complex or its interaction with excited photopigments. Additionally, Aligianis et al. (Aligianis et al. 2002) identified a consanguineous Pakistani family with six members having autosomal recessive achromatopsia. The deduced frameshift mutation in exon 7 of *GNAT2* is different from the one described by Kohl et al. (Kohl et al. 2002), suggesting that the complete spectrum of *GNAT2* mutations causing achromatopsia is not yet known. Additionally, it is important to note that not all mutations in what would appear to be "important" *GNAT2* domains cause disease. Although lysine 270 of GNAT2 has been suggested by modeling to play a key role in fixing the purine ring of GTP in the nucleotide binding cleft of GNAT2, when Pina et al. (Pina et al. 2004) examined the phenotype of a K270 deletion in homozygous carriers, segregation analysis showed that the deletion is not co-inherited with the disease phenotype and, therefore, is not the disease causing mutation. Presumably, GNAT2 function is not altered by K270 deletion possibly because of a compensatory effect of K271. Thus, GNAT2 is able to structurally and functionally tolerate a K270 deletion as shown by its ability to support normal cone function.

2.3 *CNG* achromatopsia

Cones like rods rely on cyclic nucleotide gated (CNG) channels for membrane hyperpolarization and signal transduction upon light absorption. Heterotetrameric cation channels in rods and cones form a central pore, with the occupancy of cGMP binding sites on each subunit regulating pore formation. Six genes control CNG expression in mammals, four alpha subunits (CNGA1-4) and two beta subunits (CNGB1 and CNGB3) (Bradley et al 2001). CNGA subunits can form functional homomeric proteins, while CNGB subunits must be associated with CNGA subunits to form functional channels (Kaupp and Seifert 2002). Two classes of CNGA subunits are found in vertebrate retinas: CNGA1 is expressed in rod outer segments and CNGA3 is expressed in cones. The latter is also expressed in sperm, kidney, cardiac and brain cells. Cone CNG channels consist of two A3 subunits and two B3 subunits (Bradley, Reisert and Frings 2005; Peng; Rich and Varnum 2004). In humans, cone photoreceptor function loss due to *CNGB3* gene mutations is known as achromatopsia 1 (Khan et al, 2007). Achromatopsia 2 is caused by *CNGA3* gene mutation. In European populations about 25% of patients with complete achromatopsia have *CNGA3* mutations (Kaupp and Seifert 2002; Kohl et al 2005), and another 50% have *CNGB3* mutations (Wissinger et al 2001)

2.4 Achromatopsia gene therapy

Thus far mutations causing achromatopsia disrupt either G-protein signaling (*GNAT2*) or cGMP gated cation channel function (*CNGB3* and *CNGA3*). Since these mutations appear to result in a relatively stationary cone phenotype and therefore human achromats do not generally experience severe retinal degeneration as is seen with other diseases such as Leber's Congenital Amaurosis. However, recent adaptive optics analysis of achromats with *CNGB3* mutation suggests foveal structural abnormalities (Carroll, Choi and Williams, 2008). Taken together, achromatopsia is a potentially viable candidate for gene therapy where animal models exist for all three major genetic forms of disease in which to test this hypothesis.

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3.1 The mutant Gnat2 mouse and gene therapy

The *Gnat2*^{cpfl3} mouse carries a recessive mutation in its cone α-transducin gene that results in cone mediated vision loss, little or no cone-mediated ERG and poor visual acuity, all of which are similar to the corresponding human form of achromatopsia (Chang et al 2006; Alexander et al 2007). Homozygous *Gnat2*^{cpfl3} mice were treated with a single subretinal injection of an AAV serotype 5 vector (4×10^{10} vector genome containing particles) carrying a wild type mouse *Gnat2* cDNA under control of a human red cone opsin promoter that targets vector transgene expression to cones in mice (Alexander et al 2007). In treated eyes, light-adapted (cone specific) ERG responses were stably restored to within the normal amplitude range for at least 7 months (Alexander et al 2007). Cone ERG amplitudes in untreated eyes remained undetectable. Furthermore, visual acuity was restored to normal levels as deduced through optomotor behavioral testing. These encouraging results suggest that long term, effective cone-targeted therapy is possible, providing a basis for treating a variety of related diseases. Additional testing has revealed that treated eyes also respond to cone isolating flicker ERG stimuli more robustly than untreated contralateral eyes (Figure 3.1) further confirming the efficacy to cone-target gene therapy in this model of achromatopsia.

3.2 The *Cnga3* mutant mouse and gene therapy

Recently a new mouse with a cone function loss, *cpfl5* (Cone Photoreceptor Function Loss 5) mice, that has an ocular phenotype similar to human achromatopsia was discovered in The Jackson Laboratory. *Cpfl5* is a naturally occurring mouse model of autosomal recessive achromatopsia as defined by a missense mutation in exon 5 of the *Cnga3* gene (Hawes et al. 2006). Functional studies found no cone ERG response. Histological and immunohistochemical analysis revealed a migration of cone cell bodies into the outer plexiform layer of the retina from as early as postnatal week 3 suggesting early pathogenesis.

To test whether AAV-mediated *Cnga3* gene therapy could restore cone function to *cpfl5* mice, we delivered an AAV vector encoding the wild type mouse *Cnga3* gene driven by a human blue cone promoter (HB570) that preferentially targeted transgene expression to cones to *cpfl5* mice. One μl of AAV5-HB570-*Cnga3* vector (1×10^{10} genome containing viral particles) was injected subretinally into one eye of 10 *cpfl5* mice and the untreated contralateral eyes were used as controls. Treatment was at postnatal day 14 (P14) before the cone degeneration initiated. Dark- and light-adapted ERGs were recorded periodically from 3 to 10 weeks after injections. In the treated eyes, measurable light-adapted ERG signals were observed at 3 weeks after vector administration. This restored light-adapted ERGs remained stable for at least 2 months after treatment (Figure 3.2) with b-wave amplitudes about half of that recorded in normal *C57BL/6J* mice. In the contralateral untreated eyes, cone-driven ERGs remained unrecordable. Dark-adapted ERG analysis confirmed that the rod function is normal and unaffected by vector treatment in *cpfl5* mice at this age (Figure 3.2).

3.3 The *Cngb3* mutant dog and gene therapy

One autosomal recessive canine disease that occurs naturally in the Alaskan Malamute has been found to be related to *CNGB3* mutations. Its phenotype is similar to human achromatopsia, and is characterized by day-blindness and absence of retinal cone function (Sidjanin et al. 2002). Since *CNGB3* mutations account for 50% of human achromatopsia, this dog provides a valuable large animal model for exploring disease mechanisms and evaluating potential genetic therapeutic intervention (Sidjanin et al. 2002).

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AAV-mediated gene therapy in this canine model of achromatopsia has achieved major therapeutic effect (Komaromy, 2008). ERG restoration has been observed and maintained for over 14 months in the *CNGB3* Malamute following a subretinal injection of an AAV5 vector containing human *CNGB3* cDNA controlled by a truncated human red cone opsin promoter (Komaromy, 2008).

4.1 Prospects for achromatopsia gene therapy

AAV mediated gene transfer of the corresponding wild type gene corrects cone functional deficiencies in two mouse models and one dog model representing all three (*Gnat3,Cnga3 and CNGB3*) genetic forms of human achromatopsia. Such intervention effectively restores cone system function as demonstrated by ERGs and/ or by visually elicited behavior. These data suggests achromatopsia may be a viable candidate for gene-based therapy.

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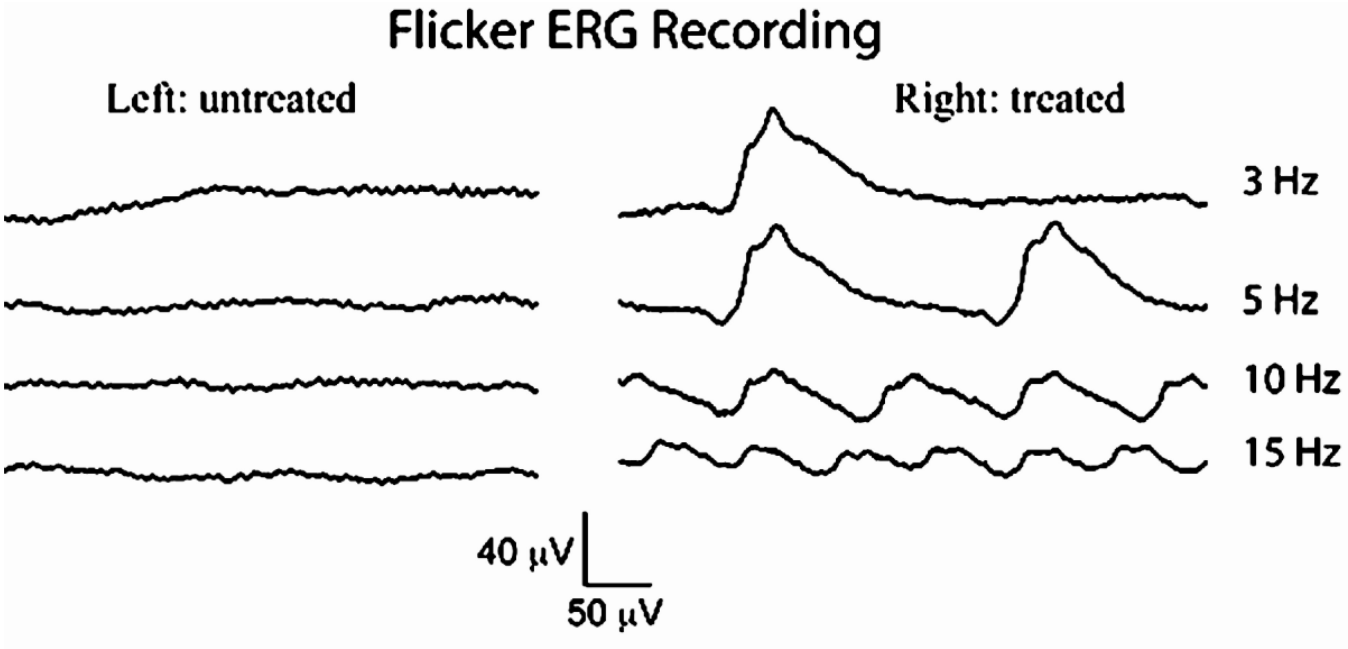

Figure 3.1.

Light-adapted flicker ERGs recorded from a *cpfl3* mouse (homozygous recessive Gnat2 mutation) at 7 months after subretinal injection of AAV vector. The right eye was vector treated and the left eye was untreated as a control. For the flicker ERG series, mice were exposed to flashes of 2.5 cd·s/m^2 at 3, 5, 10, and 15 Hz in the presence of a 30 cd · s/m^2 background light.

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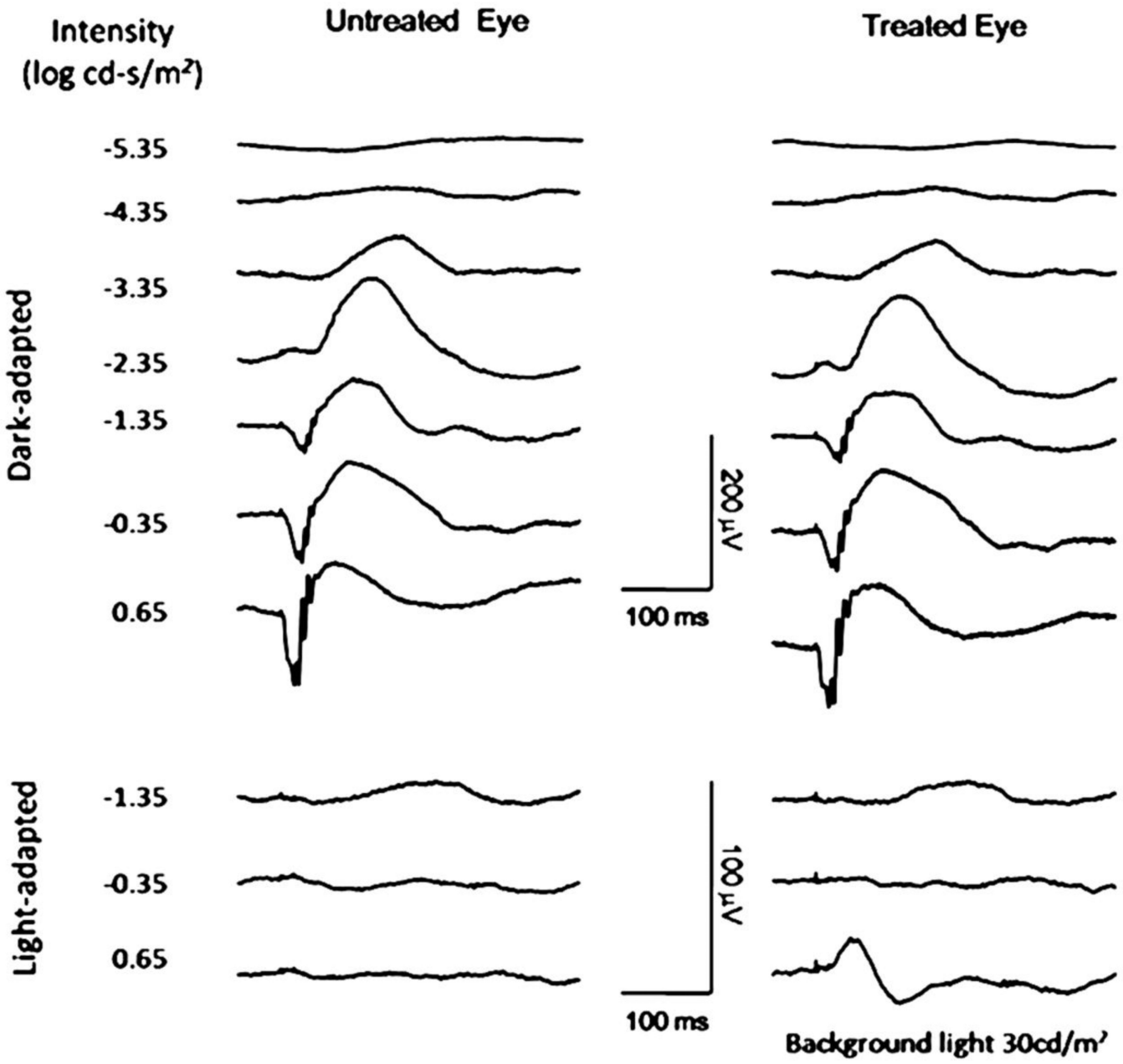

Figure 3.2.

Subretinal administration of AAV5-HB570-cnga3 vector restores the cone-driven function in *cpfl5* mouse. Representative ERGs recorded at 2 months after treatment at P14. The left column shows ERGs of an untreated control eye and the right column shows ERGs of the contralateral vector treated eye. Comparison between eyes demonstrates that gene transfer restored cone-driven function. Dark-adapted ERGs of the two eyes are comparable, suggesting the treatment had minimal effects on normal rod-driven ERGs in these mice.